system and method for performing PCR rapidly and for simultaneously monitoring the reaction. Still further, the present invention also provides a system and method for performing PCR rapidly and also continuously monitoring the reaction while it is ongoing and for adjusting the reaction parameters while the reaction is ongoing.

Information regarding an On-line DNA Analysis System with Rapid Thermal Cycling is found in U.S. Patent Application serial no. 08/381,703 filed January 31, 1995 which is now incorporated herein in its entirety.

The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

What is claimed and desired to be secured by United States Letters Patent is:

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1. A method of subjecting at least a first biological sample to rapid thermal cycling, the method comprising the steps of:

(a) placing the first biological sample in at least a first container;

(b) raising the temperature of the first biological sample from a first temperature (T_1) to a second temperature (T_2) in less than a number of second(s) equal to a value of T_2 minus T_1, expressed in the cenitgrade scale;

(c) holding the first biological sample at a temperature at least as great as the second temperature for not more than a first holding period, the first holding period being not greater than ten seconds;

(d) lowering the temperature of the first biological sample from the second temperature to at least the first temperature in less than a number of second(s) equal to a value of T_2 minus T_1, expressed in the cenitgrade scale; and

(e) holding the first biological sample at a temperature at least as low as the first temperature for

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not more than a second holding period, the second holding period being not greater than twenty seconds.

2. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of placing the first biological sample in at least a first container comprises the step of placing a volume of the first biological sample in the first container, the volume of the first biological sample being not greater than about 10,000 $\mu\ell$.

3. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of placing the first biological sample in at least a first container comprises the step of placing the first biological sample in the first container having a volume which is not greater than about 10,000 $\mu\ell$.

4. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of placing the first biological sample in at least a

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first container comprises the step of placing a volume of the first biological sample in the first container being at least partially fabricated from glass.

5. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of raising the temperature of the first biological sample comprises raising the temperature of the first biological sample from a first temperature (T_1) to a second temperature (T_2) in less than a number of second(s) equal to a value of $\frac{T_2 - T_1}{4}$.

6. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of raising the temperature of the first biological sample comprises raising the temperature of the first biological sample from a first temperature (T_1) to a second temperature (T_2) in less than a number of second(s) equal to

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a value of $\frac{T_2 - T_1}{10}$.

7. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of raising the temperature of the first biological sample comprises the step of raising the temperature of the first biological sample from a first temperature to a second temperature at a first rate at least as great as 1°C per second at a first rate at least as great as 1°C per second, the first temperature being at least 20°C different than the second temperature.

8. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of raising the temperature of the first biological sample comprises the step of raising the temperature of the first biological sample from a first temperature to a second temperature at a first rate at least as great as 10°C per

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second, the first temperature being at least 20°C different than the second temperature.

9. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of raising the temperature of the first biological sample comprises the step of raising the temperature of the first biological sample from a first temperature to a second temperature at a first rate at least as great as 20°C per second.

10. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first and second holding periods are not greater than about ten seconds.

11. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first and second holding periods are not greater than about one second.

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12. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 further comprising the step of the placing a plurality of biological samples in a plurality of containers.

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13. A system for performing PCR and monitoring the reaction in real time during temperature cycling comprising:

a sample container for holding a PCR sample, the sample container comprising an optically clear material holding less than 1 milliliter of a sample, the sample container having a first side, a second side, and an end;

means for positioning the PCR sample in a monitoring position;

means for heating the PCR sample;

means for cooling the PCR sample;

control means for repeatedly operating the means for heating and the means for cooling to subject the PCR sample to thermal cycling;

means for optically exciting the sample to cause the sample to fluoresce; and

means for detecting the fluorescence of the excited sample.

14. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 further comprising:

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means for determining at least one reaction parameter in accordance with the detected fluorescence; and

means for adjusting the control means in accordance with the reaction parameter.

15. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 14 further comprising means for adjusting the control means in accordance with the reaction parameter.

16. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 15 further comprising a control mechanism which adjusts the operation of the means for heating and the means for cooling to alter the times the means for heating and the means for cooling operate in accordance with the reaction parameter.

17. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 15 further comprising a control mechanism which adjusts

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the operation of the means for heating and the means for cooling to alter the rate at which the biological sample is heated and cooled in accordance with the reaction parameter.

18. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the sample container comprises a container fabricated at least partially from glass and having a volume not greater than about 10,000 $\mu\ell$.

19. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for positioning the PCR sample in a monitoring position comprises a rotatable carousel.

20. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 further comprising means for positioning the means for optically exciting the sample and the means for detecting the fluorescence of excited sample to optimize the fluorescence which is detected.

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21. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for heating the PCR sample comprises a forced air heater.

22. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for cooling comprises an air movement mechanism which transports ambient air to the sample container.

23. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the control means comprises a microprocessor.

24. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for optically exciting the sample comprises a photo emitter structure positioned so that the radiation emitted therefrom impinges the side of the sample container.

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25. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 24 wherein means for detecting the fluoresce of the excited sample comprises a photo detector structure positioned so that the radiation emitted from the side of the sample container is detected.

26. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for optically exciting the sample comprises a photo emitter structure positioned so that the radiation emitted therefrom impinges the end of the sample container.

27. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 26 wherein the means for detecting the fluoresce of the excited sample comprises a photo detector structure positioned so that the radiation emitted from the end of the sample container is detected.

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28. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for determining at least one reaction parameter in accordance with the detected fluoresce comprises means for determining at least one reaction parameter selected from the group consisting of: product melting temperature, product melting time, product reannealing temperature, product reannealing time, probe melting temperature, probe melting time, primer annealing/extension temperature, and primer annealing/extension time.

29. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the control means comprises means cooling the sample when the means for detecting the fluorescence of the excited sample detects that the product is completely melted.

30. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the control means comprises means for heating the sample when the means for detecting the fluorescence of

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the excited sample detects no more product generation.

31. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for optically exciting is positioned to interact with the first side of the sample container and the means for detecting the fluorescence is positioned to interact with the second side of the sample container.

32. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 13 wherein the means for optically exciting is positioned to interact with the end of the sample container and the means for detecting the fluorescence is positioned to interact with the end of the sample container.

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33. A system for performing PCR and monitoring the reaction in real time during temperature cycling comprising:

a plurality of sample containers for holding a plurality of PCR samples, the sample container comprising an optically clear capillary tube holding less than 1 milliliter of a sample having a sealed end and an open end with a sealable closure on another end;

means for holding a plurality of sample containers, the means for holding comprising a rotatable carousel holding the sample containers;

means for forcing hot fluid into contact with the plurality of sample containers;

means for forcing cool fluid into contact with the plurality of sample containers;

means for repeatedly operating the means for forcing hot fluid and the means for forcing cool fluid to subject the PCR samples to thermal cycling;

means for optically exciting the sample to cause the sample to fluoresce;

means for detecting the fluorescence of the excited sample at both a first wavelength and a second

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wavelength; and

means for determining at least one reaction parameter in accordance with the detected fluoresce and displaying the reaction parameter in a visually perceptible manner in real time.

34. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 33 further comprising means for adjusting the means for repeatedly operating in accordance with the reaction parameter such that the reaction is adjusted in real time.

35. A system for performing PCR and monitoring the reaction in real time during temperature cycling as defined in claim 33 wherein the means for determining at least one reaction parameter in accordance with the detected fluoresce and displaying the reaction parameter in a visually perceptible manner in real time comprises means for determining a reaction parameter selected from the group consisting of denaturation temperature and time, primer annealing temperature and time, probe annealing temperature

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and time, enzyme extension temperature and time, and number of cycles.

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36. A method of performing nucleic acid amplification comprising the steps of:

providing a biological sample;

raising the temperature of the sample to a first temperature;

begin cooling the sample within a period of time not greater than a first period, the first period being equal to one second;

lowering the temperature of the sample to a second temperature, the second temperature being lower than the first temperature;

keeping the sample at the second temperature for not more than the first period; and

raising the temperature of the sample from the second temperature to a third temperature, the third temperature being higher than the second temperature.

37. A method of performing nucleic amplification as defined in claim 36 wherein the step of raising the temperature of the sample to a first temperature and the step of lowering the temperature of the sample to a second

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temperature is carried out at least thirty times in twenty minutes.

38. A method of performing nucleic acid amplification as defined in claim 36 wherein the first period is not greater than 0.2 seconds.

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39. A method of performing nucleic acid amplification while monitoring the progress of the amplification, the method comprising the steps of:

providing a biological sample to undergo nucleic acid amplification;

subjecting the biological sample to a temperature transition from a first temperature to a second temperature;

exciting the biological sample during the temperature transition such that radiation is emitted from the sample;

detecting the radiation which is emitted from the sample at least once during the temperature transition to determine at least one reaction parameter.

40. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of detecting the radiation comprises the step of determining the melting temperature of a product.

41. A method of performing nucleic acid amplification as

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defined in claim 39 wherein the step of detecting the radiation comprises the step of determining the melting temperature of a probe.

42. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of subjecting the biological sample to a temperature transition comprises the step of increasing the temperature of the biological sample at a rate at least as great as 2.5°C per second.

43. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of subjecting the biological sample to a temperature transition comprises the step of increasing the temperature of the biological sample at least 40°C during a period of not more than ten seconds.

44. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of exciting the biological sample during the temperature transition comprises the step of optically exciting the biological sample.

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45. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of detecting the radiation which is emitted from the sample comprises the step of optically detecting the radiation emitted from the sample.

46. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of detecting the radiation which is emitted from the sample comprises the step of detecting the radiation emitted from the sample at least twice during the transition.

47. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of detecting the radiation which is emitted from the sample comprises the step of detecting the radiation emitted from the sample at least twice during the temperature transition.

48. A method of performing nucleic acid amplification as defined in claim 39 wherein the step of subjecting the biological sample to a temperature transition comprises the step of increasing the temperature of the biological sample

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until the product in the biological sample is completely melted.

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49. A container for holding a fluidic biological sample while undergoing nucleic acid amplification, the container comprising:

a receiving portion having a first volume, the receiving portion being adapted to receive the biological sample placed therein; and

a reaction portion, the reaction portion being in fluidic communication with the receiving portion such that the biological sample placed in the receiving portion can travel to the reaction portion, the reaction portion having an internal volume not greater than a second volume, the second volume being less than the first volume and not greater than 1 milliliter and comprised of a material having a thermal conductively in the range from about 20 to about 35 in accordance with the formula $\left(\frac{cal\ cm}{cm^2 s\ degree\ C}\right) X\ 10^4$.

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50. A container as defined in claim 49 wherein the receiver portion comprises a plastic material.

51. A container as defined in claim 49 wherein the receiver portion comprises a plastic material formed in a funnel structure.

52. A container as defined in claim 49 further wherein the comprising a stopper, the stopper being removably inserted into the receiving portion.

53. A container as defined in claim 49 wherein the second volume is not greater than about 10,000 μl.

54. A container as defined in claim 49 wherein the container wherein at least a portion of the reaction portion is transparent.

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55. A system for carrying out and monitoring the progress of a biological reaction comprising:

first holding means for holding a first biological sample;

second holding means for holding a second biological sample;

transporting means for moving the first and second means for holding between a non-monitoring position to a monitoring position;

thermal cycling means for repeatedly heating and cooling the first holding means and the second holding means in both the non-monitoring position and in the monitoring position to carry out thermal cycling on both the first biological sample and the second biological sample;

monitoring means for ascertaining the biological reaction in the first means for holding and the second means for holding when the biological sample is in the monitoring position, the means for monitoring comprising means for detecting radiation emitted from the biological sample; and

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controlling means for controlling the operation of the transporting means, thermal cycling means, and the monitoring means such that the progress of the biological reaction is detected as thermal cycling occurs.

56. A system for carrying out and monitoring the progress of a biological reaction as defined in claim 55 wherein the monitoring means comprises:

an excitation source emitting excitation radiation;

means for directing the excitation radiation to the monitoring position such that the sample located at the monitoring position emits radiation;

means for converting the emitted radiation to an electrical signal;

means for processing the electrical signal to arrive at a reaction parameter;

means for displaying the reaction parameter; and

means for recording the reaction parameter.

57. A system for carrying out and monitoring the progress of a biological reaction as defined in claim 56

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wherein the reaction parameter is selected from the group consisting of denaturation temperature and time, primer annealing temperature and time, probe annealing temperature and time, enzyme extension temperature and time, and number of cycles.

58. A system for carrying out and monitoring the progress of a biological reaction as defined in claim 56 wherein:

the excitation source comprises a photo-emitting source, the photo-emitting source selected from the group consisting of a xenon lamp and a light emitting diode;

the means for converting the emitted radiation to an electrical signal comprises a photo-detection device, the photo-detection device selected from the group consisting of a photo-multiplier tube and a photo-diode; and

the means for processing the electrical signal to arrive at a reaction parameter comprises a microprocessor.

59. A system for carrying out and monitoring the

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progress of a biological reaction as defined in claim 58 wherein the means for converting the emitted radiation to an electrical signal comprises a first photo-detection device, the first photo-detection device is selected from the group consisting of a photo-multiplier tube and a photo-diode and a second photo-detection device, the first photo-detection device and the second photo-detection device selected from the group consisting of a photo-multiplier tube and a photo-diode.

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60. A method of performing nucleic acid amplification comprising the steps of:

(a) providing a biological sample to undergo nucleic acid amplification, the biological sample including a substance which emits radiation at a first wavelength which is related to the progress of the nucleic acid amplification;

(b) adjusting the temperature of the sample over a first range including a first temperature and a second temperature, the second temperature being different than the first temperature;

(c) detecting the radiation emitted by the biological sample a plurality of times when the temperature of the sample is within the first range;

(d) adjusting the temperature of the sample over a second range including the second temperature and a third temperature, the third temperature being different than the second temperature; and

(e) detecting the radiation emitted by the biological sample a plurality of times when the temperature of the sample is within the second range.

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61. A method of performing nucleic acid amplification and monitoring the amplification reaction as set forth in claim 60 further comprising the step of:

(f) adjusting at least one parameter of the amplification reaction in accordance with the radiation detected in step (c) or (e).

62. A method of performing nucleic acid amplification and monitoring the amplification reaction as set forth in claim 60 wherein the step of adjusting the temperature of the sample over a first range comprises the step of ceasing to adjust the temperature of the sample over a first range when product melting is substantially complete.

63. A method of performing nucleic acid amplification and monitoring the amplification reaction as set forth in claim 60 wherein the step of adjusting the temperature of the sample over a second range comprises the step of ceasing to adjust the temperature of the sample over a second range when primer annealing substantially ceases.

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64. A method of performing nucleic acid amplification and monitoring the amplification reaction as set forth in claim 60 further comprising the step of adjusting the temperature of the sample over a third range and ceasing to adjust the temperature of the sample over the third range when product accumulation reaches an effective level.

65. A method of performing nucleic acid amplification and monitoring the amplification reaction as set forth in claim 60 wherein the step of detecting the radiation emitted by the biological sample a plurality of times comprises the step of detecting the radiation emitted from the biological sample in the range from about 400 nm to about 800 nm.

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66. A carousel for holding a sample and delivering it to a sample vessel for analysis, said carousel comprising

a disc having a top surface, a bottom surface, an outer edge extending therebetween, a sample receiving port in the top surface, a sample vessel port in the outer edge and a sample passageway communicating with said sample receiving port and the sample vessel port, said sample vessel port and passageway formed for receiving and fixing a sample vessel to the disc.

67. The carousel of claim 66 further comprising a closure for the sample receiving port.

68. The carousel of claim 66 wherein the passageway includes a barrier that prevents a liquid sample delivered through the sample receiving port from flowing to the sample vessel port absent a biasing force on said liquid sample.

69. The carousel of claim 66 or 68 further comprising a sample vessel received in the sample vessel port.

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70. The carousel of claim 69 wherein the sample vessel is a capillary tube having a volume to surface area of greater than 1mm.

71. The carousel of claim 66 wherein the passageway communicates with the sample vessel port, the sample receiving port, and at least one additional port formed in said top surface.

72. The carousel of claim 71 wherein the passageway includes a barrier that prevents a liquid sample delivered through the sample receiving port from flowing to the sample vessel port absent a biasing force on said liquid sample.

73. The carousel of claim 72 wherein the passageway includes an additional barrier that prevents a liquid delivered through the additional port from flowing into the sample vessel port absent a biasing force on said liquid sample.

74. The carousel of claim 72 or 73 further comprising a

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predetermined reagent mixture in the passageway between the sample receiving port and the sample vessel port.

75. The carousel of claim 72 or 73 further comprising a sample vessel received in the sample vessel port.

76. The carousel of claim 74 further comprising a sample vessel received in the sample vessel port.

77. The carousel of claim 76 wherein the sample vessel is a capillary tube having a volume to surface area of greater than 1mm.

78. The carousel of claim 75 wherein the sample vessel is a capillary tube having a volume to surface area of greater than 1mm.

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79. A device for monitoring the fluorescence of a sample held within a sample vessel, said device comprising

a chamber;

a sample vessel holder for holding the sample vessel, said sample vessel comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1mm;

a light emitting source mounted in said chamber and positioned to illuminate the sample vessel along an axis substantially parallel to a wall along the second dimension of the vessel; and

a light detector mounted in said chamber and positioned to measure fluorescence from the sample vessel along an axis substantially parallel to a wall along the second dimension of the vessel.

80. The device of claim 79 wherein the sample vessel holder comprises a carousel for holding a plurality of

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capillary tubes, said carousel being rotatably mounted in said chamber, said device further comprising

a stepper motor for rotating said carousel; and

means for coupling said carousel to said motor.

81. The device of claims 79 or 80 wherein the chamber is further provided with a heater and a fan mounted in said device in air flow communication with the chamber and a controller therefor for rapidly cycling the temperature of the chamber .

Substitute Sheet (Rule 26)

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82. A device for conducting PCR reactions said device comprising

a chamber;

a heater and a fan mounted in said device and in air flow communication with the chamber;

carousel for holding a plurality of sample vessels, said carousel being rotatably mounted in said chamber;

said sample vessels comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1mm;

a light emitting source mounted in said chamber and positioned to illuminate at least one of the sample vessels along an axis substantially parallel to a wall along the second dimension of the vessel; and

a light detector mounted in said chamber and positioned to measure fluorescence from at least one of the sample vessels along an axis substantially parallel to a wall along the second dimension of the vessel.

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83. A handling system for a biological sample comprising a vessel having a sample delivery port and a funnel cap for filling said vessel, said vessel comprising walls of an optically transparent material, said walls defining a volume wherein the ratio of vessel volume to external vessel surface area is less than 1mm, said funnel cap having a first sample receiving port and a second sample transfer port and means for releasably fixing the funnel cap on the vessel so that the sample transfer port of the funnel cap and the sample delivery port of the vessel are in alignment.

84. The sample handling system of claim 83 further comprising a plug for frictional fit sealing engagement with the sample receiving port of the funnel cap.

85. The sample handling system of claim 83 wherein the vessel is a capillary tube.

86. The sample handling system of claim 83 wherein the

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ratio of the volume of the vessel to its external surface area is less than 0.25mm.

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87. A system for performing PCR and monitoring the reaction in real time comprising:

a chamber;

a heater and a fan mounted in said device and in air flow communication with the chamber and a controller for cycling the temperature in the chamber according to initial predefined temperature and time parameters;

a carousel for holding a plurality of sample vessels said carousel being rotatably mounted in said chamber, said sample vessels comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1mm;

a light emitting source mounted in said chamber and positioned to illuminate at least one of the sample vessels along an axis substantially parallel to a wall along the second dimension of the vessel; and

a light detector mounted in said chamber and positioned to measure fluorescence from at least one of

the sample vessels along an axis substantially parallel to a wall along the second dimension of the vessel;

means for displaying the status of the reaction based detected fluorescence.

88. The system of claim 87 further comprising means for adjusting the controller such that one or more reaction parameters the reaction is adjusted in real time.

89. The system of claim 87 or 88 wherein the carousel comprises:

a disc having a top surface, a bottom surface, an outer edge extending therebetween, a sample receiving port in the top surface, a sample vessel port in the outer edge, and a sample passageway communicating with said sample receiving port and the sample vessel port, said sample vessel port and passageway formed for receiving and fixing a sample vessel to the disc.

90. The system of claim 87 or 88 wherein the sample vessels are capillary tubes having an inner diameters ranging

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from about 0.02mm to about 1.0mm.

91. The system of claim 89 wherein the passageway of the carousel includes a barrier that prevents a liquid sample delivered through the sample receiving port from flowing to the sample vessel port absent a biasing force on said liquid sample.

92. The system of claim 89 further comprising a motor for rotating the carousel to provide a biasing force on a liquid sample delivered through the sample receiving port.

Exhibit 2006

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93. A method for adding a liquid sample to a capillary sample vessel, said method comprising the steps of:

selecting a carousel for receiving said sample and holding said sample vessel, said carousel comprising a disc having a top surface, a bottom surface and an outer edge extending therebetween, a sample receiving port in the top surface, a sample vessel port in the outer edge and a sample passageway communicating with said sample receiving port and the sample vessel port, said sample vessel port and passageway formed for receiving and fixing a sample vessel to the disc;

delivering the liquid sample into the sample receiving port;

positioning a capillary sample vessel into the sample vessel port; and

rotating the carousel to deliver the sample into the capillary sample vessel.

94. The method of claim 93 further comprising the step of adding a predetermined mixture to the sample vessel before positioning it into the sample vessel port.

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95. The method of claim 93 further comprising the step of positioning a predetermined mixture in the sample passageway before delivering the liquid sample into the sample receiving port.

96. The method of claim 94 or 95 wherein the predetermined mixture comprises reagents for conducting analysis of the liquid sample.

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97. A method for adding a liquid sample to a capillary sample vessel, said method comprising the steps of:

selecting a carousel for receiving said sample and holding said sample vessel, said carousel comprising a disc having a top surface, a bottom surface and an outer edge extending therebetween, a sample receiving port in the top surface, a sample vessel port in the outer edge and a sample passageway communicating with said sample receiving port and the sample vessel port, wherein the passageway includes a barrier that prevents a liquid sample delivered through the sample receiving port from flowing to the sample vessel port absent a biasing force on said liquid sample, said sample vessel port and passageway formed for receiving and fixing a sample vessel to the disc;

delivering the liquid sample into the sample receiving port;

positioning a capillary sample vessel into the sample vessel port; and

rotating the carousel to deliver the sample into the capillary sample vessel.

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98. The method of claim 97, wherein the passageway communicates with the sample vessel port, the sample receiving port and at least one additional port formed in said top surface, said method comprising the additional step of delivering a second liquid into the passageway through the additional port before the carousel is rotated, so that when the carousel is rotated to provide a biasing force on the liquid sample and the second liquid, they are mixed as they are delivered into the capillary vessel.

99. The method of claim 98 wherein the passageway includes an additional barrier that prevents the second liquid from flowing to the sample vessel port absent a biasing force on said second liquid delivered into the passageway through the additional port.

100. The method of claim 99 wherein the additional barrier prevents the second liquid sample from flowing to the sample vessel port when the carousel is rotated a predetermined rate of rotation that delivers the first sample to the capillary sample vessel, said method comprising the

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additional step of rotating the carousel at a second higher rate of rotation that delivers the second liquid into the capillary vessel.

101. The method of claims 97, 98, 99 or 100 further comprising the step of adding a predetermined mixture to the sample vessel before positioning a capillary sample vessel into the sample vessel port.

102. The method of claims 97, 98, 99 or 100 further comprising the step of coupling the carousel to a stepper motor for rotating said carousel.

103. The method of claims 101 wherein the predetermined mixture comprises reagents for conducting analysis of the sample.

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104. A system for detecting the presence of a target nucleic acid sequence in a sample, said system comprising:

a pair of oligonucleotide probes that hybridize to adjacent regions of the target nucleic acid sequence, one of said probes being labeled with an acceptor fluorophore and the other probe labeled with a donor fluorophore of a fluorescence energy transfer pair, wherein the donor fluorophore emission and the acceptor fluorophore absorption overlap less than 25%, and the acceptor fluorophore has a peak extinction coefficient greater than 100,000 $M^{-1}cm^{-1}$ and upon hybridization of the two probes with the target sequence, the donor and acceptor fluorophores are within 15 nucleotides of one another.

105. The system of claim 104 wherein the resonance energy transfer pair comprises fluorescein as the donor.

106. The system of claim 105 wherein Cy5 is the acceptor fluorophore.

107. The system of claim 104 wherein the donor and

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acceptor fluorophores are within 0-5 nucleotides of one another when the probes are hybridized to the target nucleic acid sequence.

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108. A method for enhancing detection and efficiency of acquisition of fluorescence in a sample comprising a fluorophore, said method comprising the steps of:

placing the sample in a sample vessel, said sample vessel comprising an optically transparent material and walls defining a volume having at least first and second dimensions wherein the first dimension is less than the second dimension and wherein the ratio of volume to external surface area of the vessel is less than 1 mm; and

detecting the fluorescence along an axis substantially parallel to a wall along the second dimension of the vessel.

109. The method of claim 108 wherein the fluorescence is induced by fluorophore-excitatory illumination of the sample and the method further comprises illuminating the sample along an axis substantially parallel to a wall along the second dimension of the vessel.

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110. The method of claim 108 wherein the fluorescence is induced by fluorophore-excitatory illumination of the sample and the method further comprises illuminating the sample along the fluorescence detection axis.

111. The method of claim 108, 109, or 110 wherein the ratio of the volume to the external surface area of the vessel is less than 0.5mm.

112. The method of claim 108, 109, or 110 wherein the ratio to the volume to external surface area of the vessel is less than 0.25mm.

113. The method of claim 108, 109, 110 or 111 wherein the vessel is a capillary tube.

114. The method of claim 108, 109, 110 or 111 wherein the vessel comprises two spaced-apart plates or sheets sealed at their edges.

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115. The method of claim 108 wherein the fluorescence is detected along an axis through a wall of the vessel having the smallest surface area.

116. The method of claim 115 wherein the sample is illuminated along the fluorescence detection axis.

117. The method of claim 115 wherein the vessel is a capillary tube and fluorescence is detected along an axis through the bottom of the capillary tube.

add C'
Cancel D13

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Add G3

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